Urinary Peptides in Hepatocellular Carcinoma

Subjects: Pathology

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Analysis of the urine samples was performed by capillary electrophoresis (CE) coupled to mass spectrometry (MS). Peptide sequences were obtained and 31 specific peptide markers for hepatocellular carcinoma (HCC) were identified and further integrated into a multivariate classification model. The discovered urinary peptides offered a potential noninvasive tool for diagnosis and prognosis of hepatocellular carcinoma.

hepatocellular carcinoma | capillary electrophoresis mass spectrometry | urinary peptides | diagnosis | prognosis

1. Introduction

Hepatocellular carcinoma (HCC) incidence is increasing worldwide, and it is the third most frequent cause of cancer related death globally [1]. HCC is more frequent in males than females and usually occurs at older ages (>60 years). Most patients who develop HCC are asymptomatic in the early stages of disease, with features of abdominal pain, abdominal mass and deranged liver function tests (LFTs) infrequently present. HCC is typically identified clinically when patients affected by liver cirrhosis (LC) develop sudden hepatic decompensation with features such as ascites, jaundice, hepatic encephalopathy, or variceal bleeding [2][3][4].

HCC is the end result of progressive liver fibrosis and liver cirrhosis (LC). Various causes can lead to chronic liver injury provoking an inflammatory response and resulting in liver fibrosis through activation of the hepatic stellate cells. At the molecular level, this activation is associated with protein changes in the liver extracellular matrix (ECM). The ECM consists of an array of various proteins that comprise the scaffolding of the liver. Morphologically liver fibrosis is characterized by an excessive deposition of collagen-rich ECM components [5].

For many years, 2D gel electrophoresis was the principal proteomic technology. It is now largely replaced by mass spectrometry detection usually connected to a preceding non-gel-based separation through liquid chromatography (LC) or capillary electrophoresis (CE) enabling multidimensional analyte detection in complex biofluids with high-resolution capacity. Due to these characteristics, mass spectrometry-based techniques are increasingly used in medical research including proteomic characterization, biomarker identification and diagnostic evaluation of liver and other tumours [6][7][8][9]. Capillary electrophoresis mass spectrometry (CE-MS) has emerged in recent years as a hybrid technology using capillary electrophoresis (CE) instead of liquid chromatography for sensitive (up to 1 fmol) and high-resolution low molecular weight protein and peptide separation before mass spectrometry (MS). CE-MS does not require a sieving matrix, and it also does not depend on buffer gradients and, since no continuous

adaptation of electrospray conditions is needed for optimal ionization, separation and detection of samples can be conducted fully automated. Clinical application of the CE-MS system has been demonstrated in technical reports and previous large-scale clinical studies [10][11][12][13]. Notably, this method enables profiling of the proteomic content of body fluids, such as urine, plasma or bile, in a mass range of 0.8 to 20 kilodalton (kDa). So far, it is one of the most applicable methods for monitoring of systemic catabolic processes caused by differences in the proteolytical environment at tissue and organ sites [14][15][16].

2. Pathophysiological Implications of Urinary Peptides in Hepatocellular Carcinoma

There are no accurate diagnostic biomarkers for HCC or population-based screening. Additionally, surveillance strategies for HCC are ineffective, relying on liver ultrasound scans (USS) for the detection of nodules in LC patients, which is dependent on the quality of training of the USS operator. The role of ∝-fetoprotein (AFP) in HCC surveillance is also questionable due its poor sensitivity and is no longer recommended for routine use. HCC diagnosis relies mainly on the ability of advanced, high-resolution imaging techniques for the detection of liver lesion early arterial enhancement followed by early washout. These scans are not easily accessible and can be less accurate in detecting lesions less than 1–2 cm. The current modalities used are contrast-enhanced triphasic computed tomography (CT) and/or contrast-enhanced magnetic resonance imaging (MRI). If the scans are inconclusive, the diagnosis is then confirmed with a cytological or histopathological evaluation of the liver lesion from tissue biopsy. Treatment and prognostication of patients with HCC consider the size and number of tumour nodules and their relation to the portal vein, and the degree of liver impairment [2][3][4]. Given these factors, there is a need for non-invasive methods to identify HCC.

The HCC-31 classifier adds to the current modalities for non- or minimal-invasive HCC diagnosis. To put this in a clinical perspective, the HCC-31 performed better in comparison to AFP. HCC-31 showed sensitivity of 79.5% while the quoted literature showed that AFP usually has low sensitivity for HCC detection between 40–65% [17]. Therefore, potential use of HCC-31 is promising if further validated as substitute to AFP in aiding HCC diagnosis or as a prognostic marker.

HCC-31 utilizes a molecular pattern of 31 peptides, which are surrogate markers for differential proteolytic activity at the HCC tumor site in comparison to other cirrhotic and non-cirrhotic liver diseases. Validation of HCC-31 on an independent cross-sectional cohort of 39 HCC and 87 highly heterogeneous non-HCC liver disease patients from two clinical populations, one in England and the other in Germany, resulted in an accuracy of 83.3% of the pure classification model and 91.3% when the model was adjusted for gender and age. Moreover, HCC-31 positivity was associated with a 4-fold increased risk of death during a 500-day observational period providing further evidence for its clinical applicability.

Collagen chains are the main components of the extracellular matrix, and their fragments are predominant in the low molecular weight fraction of the urinary proteome [18]. Various proteases are able to cleave collagen chains, most prominent are matrix metalloproteinases and cathepsins [19].

Carcinogenesis exact mechanisms are yet to be identified. However, cancer cells' metabolism involves extracellular proteolytic degradation. This mainly plays a role in cell migration, tumour growth and distant spreading in the body [20]. Therefore, investigations at the protein level (proteomics) are advantageous particularly in the case of in-depth characterization of cancer progression and invasiveness. CE-MS has demonstrated in this context a good diagnostic potential of urinary peptide biomarkers even for non-renal diseases with exosomes as the potential trans-renal carriers. These biomarkers have been identified in the context of a single type of cancer (e.g., bladder, prostate, pancreatic, renal cell carcinoma and cholangiocarcinoma) [21][22][23][24].

KLK6 is a protease that belongs to the kallikrein family of fifteen members located on chromosome 19. KLK6 was shown to be involved in many cancers' formation and progression [25][26][27][28]. In the liver, KLK6 was shown to catalyse ubiquitin, an important cellular regulatory protein involved in protein synthesis. KLK6 also was shown to induce de novo cirrhosis and was increased in HCC tissues [29]. Additionally, a study designed to check the activity of KLK6 on ECM peptides in HCC revealed that KLK6 has an upregulated activity [30].

MEP1A is a metalloproteinase that belongs to the metzincin family with the main function in intracellular transport of proteins [31]. MEP1A has been implicated in kidney, colorectal and pancreatic cancers [21][32][33]. In HCC, MEP1A was shown to promote cell proliferation, migration and invasion [34][35]. However, both the staining in cirrhosis and HCC tissues were negative but present in normal livers. This was also noted by OuYang et al. [35] on HCC tissues, where immunohistochemical MEP1A expression levels in the tumour cell cytoplasm varied widely among different HCC specimens. However, the same group showed that MEP1A was found to be elevated following analysis of the HCC tissues using quantitative real-time polymerase chain reaction compared with matched adjacent nonneoplastic tissues and non-malignant liver disease tissues. Differential regulation in this respect might occur on the protein level, e.g., by secretion of soluble MEP1A, rather than forming a membrane-bound complex within the cell or on the cell surface [36]. In addition, the presence of MEP1A in HCC tissues also demonstrated poor prognosis [35].

The predicted seven proteases in this study could also be potential sites for anti-protease treatment in HCC. An example was demonstrated in a study by Tran et al. [37]. They showed that injection of metalloproteinases (MMPs) inhibitors to HCC cell lines resulted in delaying HCC growth without treatment related toxicity. MMP inhibitors also lead to inhibition of angiogenesis and tumour necrosis. Furthermore, anti-cathepsins were found to promote cell death in a study completed on HepG2 cell lines [38]. These anti-proteases could be used through an immunotherapy approach in combination with conventional chemotherapy and/or nanoparticle based intervention.

CE-MS technology has identified an important sequence of urinary peptides related to proteolytic activity in HCC. The technology paves the way for future work on these peptides to develop a noninvasive test that could be applied early for purpose of screening, surveillance and/or diagnosis. The study was limited by the relatively small number of patients, small number of human liver tissue samples and its exploratory nature; nonetheless, it was multicentre and validated across two populations. In addition, the presentation of the predicted proteases was verified at the tissue level demonstrating that these urinary peptides are related to the HCC disease formation in the liver.

3. Conclusions

Urinary CE-MS analysis identified proteases specific to HCC. In addition, the specific HCC peptide model showed good diagnostic performance and prognostic ability in relation to outcomes.

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